Figure 1: Nucleotide (SEQ ID NO: 1) and deduced amino acid sequence of human ChemerinR (AC075748)

```
  1 M   E   D   E   D   Y   N   T   S   I   S   Y   G   D   E      15
175 ATG GAG GAT GAA GAT TAC AAC ACT TCC ATC AGT TAC GGT GAT GAA     219

 16 Y   P   D   Y   L   D   S   I   V   V   L   E   D   L   S      30
220 TAC CCT GAT TAT TTA GAC TCC ATT GTG GTT TTG GAG GAC TTA TCC     264

 31 P   L   E   A   R   V   T   R   I   F   L   V   V   V   Y      45
265 CCC TTG GAA GCC AGG GTG ACC AGG ATC TTC CTG GTG GTG GTC TAC     309

 46 S   I   V   C   F   L   G   I   L   G   N   G   L   V   I      60
310 AGC ATC GTC TGC TTC CTC GGG ATT CTG GGC AAT GGT CTG GTG ATC     354

 61 I   I   A   T   F   K   M   K   K   T   V   N   M   V   W      75
355 ATC ATT GCC ACC TTC AAG ATG AAG AAG ACA GTG AAC ATG GTC TGG     399

 76 F   L   N   L   A   V   A   D   F   L   F   N   V   F   L      90
400 TTC CTC AAC CTG GCA GTG GCA GAT TTC CTG TTC AAC GTC TTC CTC     444

 91 P   I   H   I   T   Y   A   A   M   D   Y   H   W   V   F     105
445 CCA ATC CAT ATC ACC TAT GCC GCC ATG GAC TAC CAC TGG GTT TTC     489

106 G   T   A   M   C   K   I   S   N   F   L   L   I   H   N     120
490 GGG ACA GCC ATG TGC AAG ATC AGC AAC TTC CTT CTC ATC CAC AAC     534

121 M   F   T   S   V   F   L   L   T   I   I   S   S   D   R     135
535 ATG TTC ACC AGC GTC TTC CTG CTG ACC ATC ATC AGC TCT GAC CGC     579

136 C   I   S   V   L   L   P   V   W   S   Q   N   H   R   S     150
580 TGC ATC TCT GTG CTC CTC CCT GTC TGG TCC CAG AAC CAC CGC AGC     624

151 V   R   L   A   Y   M   A   C   M   V   I   W   V   L   A     165
625 GTT CGC CTG GCT TAC ATG GCC TGC ATG GTC ATC TGG GTC CTG GCT     669

166 F   F   L   S   S   P   S   L   V   F   R   D   T   A   N     180
670 TTC TTC TTG AGT TCC CCA TCT CTC GTC TTC CGG GAC ACA GCC AAC     714

181 L   H   G   K   I   S   C   F   N   N   F   S   L   S   T     195
715 CTG CAT GGG AAA ATA TCC TGC TTC AAC AAC TTC AGC CTG TCC ACA     759

196 P   G   S   S   S   W   P   T   H   S   Q   M   D   P   V     210
760 CCT GGG TCT TCC TCG TGG CCC ACT CAC TCC CAA ATG GAC CCT GTG     804

211 G   Y   S   R   H   M   V   V   T   V   T   R   F   L   C     225
805 GGG TAT AGC CGG CAC ATG GTG GTG ACT GTC ACC CGC TTC CTC TGT     849

226 G   F   L   V   P   V   L   I   I   T   A   C   Y   L   T     240
850 GGC TTC CTG GTC CCA GTC CTC ATC ATC ACA GCT TGC TAC CTC ACC     894

241 I   V   C   K   L   Q   R   N   R   L   A   K   T   K   K     255
895 ATC GTC TGC AAA CTG CAG CGC AAC CGC CTG GCC AAG ACC AAG AAG     939

256 P   F   K   I   I   V   T   I   I   I   T   F   F   L   C     270
```

Figure 1 Continued

```
 940 CCC TTC AAG ATT ATT GTG ACC ATC ATC ATT ACC TTC TTC CTC TGC  984

 271  W   C   P   Y   H   T   L   N   L   L   E   L   H   H   T  285
 985 TGG TGC CCC TAC CAC ACA CTC AAC CTC CTA GAG CTC CAC CAC ACT 1029

 286  A   M   P   G   S   V   F   S   L   G   L   P   L   A   T  300
1030 GCC ATG CCT GGC TCT GTC TTC AGC CTG GGT TTG CCC CTG GCC ACT 1074

 301  A   L   A   I   A   N   S   C   M   N   P   I   L   Y   V  315
1075 GCC CTT GCC ATT GCC AAC AGC TGC ATG AAC CCC ATT CTG TAT GTT 1119

 316  F   M   G   Q   D   F   K   K   F   K   V   A   L   F   S  330
1120 TTC ATG GGT CAG GAC TTC AAG AAG TTC AAG GTG GCC CTC TTC TCT 1164

 331  R   L   V   N   A   L   S   E   D   T   G   H   S   S   Y  345
1165 CGC CTG GTC AAT GCT CTA AGT GAA GAT ACA GGC CAC TCT TCC TAC 1209

 346  P   S   H   R   S   F   T   K   M   S   S   M   N   E   R  360
1210 CCC AGC CAT AGA AGC TTT ACC AAG ATG TCA TCA ATG AAT GAG AGG 1254

 361  T   S   M   N   E   R   E   T   G   M   L   *              372
1255 ACT TCT ATG AAT GAG AGG GAG ACC GGC ATG CTT TGA             1290
```

Figure 2: Amino acid sequence of human ChemerinR (371 amino acids) (SEQ ID NO 2). The seven predicted transmembrane domaines are underlined. The consensus sequence for *N*-linked glycosylation (N-X-S/T) in the N terminus is bold and the potential site of phosphorylation by PKC (S/T-X-R/K) in the C terminus is in italic.

MEDEDY**NTS**ISYGDEYPDYLDSIVVLEDLSPLEARVTRIFLVVVYSIVCFLGILGNGLVIIIAT
FKMKKTVNMVWFLNLAVADFLFNVFLPIHITYAAMDYHWVFGTAMCKISNFLLIHNMFTSVFLL
TIISSDRCISVLLPVWSQNHRSVRLAYMACMVIWVLAFFLSSPSLVFRDTANLHGKISCFNNFS
LSTPGSSSWPTHSQMDPVGYSRHMVVTVTRFLCGFLVPVLIITACYLTIVCKLQRNRLAKTKKP
FKIIVTIIITFFLCWCPYHTLNLLELHHTAMPGSVFSLGLPLATALAIANSCMNPILYVFMGQD
FKKFKVALFSRLVNALSEDTGHSSYP*SHR*SFTKMSSMNERTSMNERETGML

Figure 3: Nucleotide and deduced amino acid sequence of mouse dez (AC u79525 – SEQ ID NOs:3 and 4, respectively)

```
   1  M   E   Y   D   A   Y   N   D   S   G   I   Y   D   D   E      15
 265 ATG GAG TAC GAC GCT TAC AAC GAC TCC GGC ATC TAT GAT GAT GAG     309

  16  Y   S   D   G   F   G   Y   F   V   D   L   E   E   A   S      30
 310 TAC TCT GAT GGC TTT GGC TAC TTT GTG GAC TTG GAG GAG GCG AGT     354

  31  P   W   E   A   K   V   A   P   V   F   L   V   V   I   Y      45
 355 CCG TGG GAG GCC AAG GTG GCC CCG GTC TTC CTG GTG GTG ATC TAC     399

  46  S   L   V   C   F   L   G   L   L   G   N   G   L   V   I      60
 400 AGC TTG GTG TGC TTC CTC GGT CTC CTA GGC AAC GGC CTG GTG ATT     444

  61  V   I   A   T   F   K   M   K   K   T   V   N   T   V   W      75
 445 GTC ATC GCC ACC TTC AAG ATG AAG AAG ACC GTG AAC ACT GTG TGG     489

  76  F   V   N   L   A   V   A   D   F   L   F   N   I   F   L      90
 490 TTT GTC AAC CTG GCT GTG GCC GAC TTC CTG TTC AAC ATC TTT TTG     534

  91  P   M   H   I   T   Y   A   A   M   D   Y   H   W   V   F     105
 535 CCG ATG CAC ATC ACC TAC GCG GCC ATG GAC TAC CAC TGG GTG TTC     579

 106  G   K   A   M   C   K   I   S   N   F   L   L   S   H   N     120
 580 GGG AAG GCC ATG TGC AAG ATC AGC AAC TTC TTG CTC AGC CAC AAC     624

 121  M   Y   T   S   V   F   L   L   T   V   I   S   F   D   R     135
 625 ATG TAC ACC AGC GTC TTC CTG CTG ACT GTC ATC AGC TTT GAC CGC     669

 136  C   I   S   V   L   L   P   V   W   S   Q   N   H   R   S     150
 670 TGC ATC TCC GTG CTG CTC CCC GTC TGG TCC CAG AAC CAC CGC AGC     714

 151  I   R   L   A   Y   M   T   C   S   A   V   W   V   L   A     165
 715 ATC CGC CTG GCC TAC ATG ACC TGC TCG GCC GTC TGG GTC CTG GCT     759

 166  F   F   L   S   S   P   S   L   V   F   R   D   T   A   N     180
 760 TTC TTC TTG AGC TCC CCG TCC CTT GTC TTC CGG GAC ACC GCC AAC     804

 181  I   H   G   K   I   T   C   F   N   N   F   S   L   A   A     195
 805 ATT CAT GGG AAG ATA ACC TGC TTC AAC AAC TTC AGC TTG GCC GCG     849

 196  P   E   S   S   P   H   P   A   H   S   Q   V   V   S   T     210
 850 CCT GAG TCC TCC CCA CAT CCC GCC CAC TCG CAA GTA GTT TCC ACA     894

 211  G   Y   S   R   H   V   A   V   T   V   T   R   F   L   C     225
 895 GGG TAC AGC AGA CAC GTG GCG GTC ACT GTC ACC CGC TTC CTT TGC     939

 226  G   F   L   I   P   V   F   I   I   T   A   C   Y   L   T     240
 940 GGC TTC CTG ATC CCC GTC TTC ATC ATC ACG GCC TGC TAC CTT ACC     984

 241  I   V   F   K   L   Q   R   N   R   L   A   K   N   K   K     255
 985 ATC GTC TTC AAG CTG CAG CGC AAC CGC CTG GCC AAG AAC AAG AAG    1029

 256  P   F   K   I   I   I   T   I   I   I   T   F   F   L   C     270
1030 CCC TTC AAG ATC ATC ATC ACC ATC ATC ATC ACC TTC TTC CTC TGC    1074

 271  W   C   P   Y   H   T   L   Y   L   L   E   L   H   H   T     285
1075 TGG TGC CCC TAC CAC ACC CTC TAC CTG CTG GAG CTC CAC CAC ACA    1119

 286  A   V   P   S   S   V   F   S   L   G   L   P   L   A   T     300
1120 GCT GTG CCA AGC TCT GTC TTC AGC CTG GGG CTA CCC CTG GCC ACG    1164
```

Figure 3 Continued

```
 301  A   V   A   I   A   N   S   C   M   N   P   I   L   Y   V    315
1165 GCC GTC GCC ATC GCC AAC AGC TGC ATG AAC CCC ATT CTG TAC GTC   1209

 316  F   M   G   H   D   F   R   K   F   K   V   A   L   F   S    330
1210 TTC ATG GGC CAC GAC TTC AGA AAA TTC AAG GTG GCC CTC TTC TCC   1254

 331  R   L   A   N   A   L   S   E   D   T   G   P   S   S   Y    345
1255 CGC CTG GCC AAC GCC CTG AGT GAG GAC ACA GGC CCC TCC TCC TAC   1299

 346  P   S   H   R   S   F   T   K   M   S   S   L   N   E   K    360
1300 CCC AGT CAC AGG AGC TTC ACC AAG ATG TCG TCT TTG AAT GAG AAG   1344

 361  A   S   V   N   E   K   E   T   S   T   L   *                372
1345 GCT TCG GTG AAT GAG AAG GAG ACC AGT ACC CTC TGA               1380
```

Figure 4: Nucleotide and deduced amino acid sequence of rat G-protein coupled chemoattractant-1 (AC NM_022218 - SEQ ID Nos: 5 and 6, respectively).

```
  1 M  E  Y  E  G  Y  N  D  S  S  I  Y  G  E  E      15
  1 ATG GAG TAC GAG GGT TAC AAC GAC TCC AGC ATC TAC GGT GAG GAG     45

 16 Y  S  D  G  S  D  Y  I  V  D  L  E  E  A  G      30
 46 TAT TCT GAC GGC TCG GAC TAC ATC GTG GAC TTG GAG GAG GCG GGT    90

 31 P  L  E  A  K  V  A  E  V  F  L  V  V  I  Y      45
 91 CCA CTG GAG GCC AAG GTG GCC GAG GTC TTC CTG GTG GTA ATC TAC   135

 46 S  L  V  C  F  L  G  I  L  G  N  G  L  V  I      60
136 AGC TTG GTG TGC TTC CTC GGG ATC CTA GGC AAT GGC CTG GTG ATT   180

 61 V  I  A  T  F  K  M  K  K  T  V  N  T  V  W      75
181 GTC ATC GCC ACC TTC AAG ATG AAG AAG ACG GTG AAC ACC GTG TGG   225

 76 F  V  N  L  A  V  A  D  F  L  F  N  I  F  L      90
226 TTT GTC AAC CTG GCC GTG GCT GAC TTC CTG TTC AAC ATC TTC TTG   270

 91 P  I  H  I  T  Y  A  A  M  D  Y  H  W  V  F     105
271 CCC ATC CAC ATC ACC TAT GCC GCT ATG GAC TAC CAC TGG GTG TTC   315

106 G  K  A  M  C  K  I  S  S  F  L  L  S  H  N     120
316 GGG AAA GCC ATG TGC AAG ATT AGT AGC TTT CTG CTA AGC CAC AAC   360

121 M  Y  T  S  V  F  L  L  T  V  I  S  F  D  R     135
361 ATG TAC ACC AGC GTC TTC CTG CTC ACT GTC ATC AGC TTC GAC CGC   405

136 C  I  S  V  L  L  P  V  W  S  Q  N  H  R  S     150
406 TGC ATC TCC GTG CTC CTC CCC GTC TGG TCC CAG AAC CAC CGC AGC   450

151 V  R  L  A  Y  M  T  C  V  V  V  W  V  W  L     165
451 GTG CGT CTG GCC TAC ATG ACC TGC GTG GTT GTC TGG GTC TGG CTT   495

166 S  S  E  S  P  P  S  L  V  F  G  H  V  S  T     180
496 TCT TCT GAG TCT CCC CCG TCC CTC GTC TTC GGA CAC GTC AGC ACC   540

181 S  H  G  K  I  T  C  F  N  N  F  S  L  A  A     195
541 AGC CAC GGG AAG ATA ACC TGC TTC AAC AAC TTC AGC CTG GCG GCG   585

196 P  E  P  F  S  H  S  T  H  P  R  T  D  P  V     210
586 CCC GAG CCT TTC TCT CAT TCC ACC CAC CCG CGA ACA GAC CCG GTA   630

211 G  Y  S  R  H  V  A  V  T  V  T  R  F  L  C     225
631 GGG TAC AGC AGA CAT GTG GCG GTC ACC GTC ACC CGC TTC CTC TGT   675

226 G  F  L  I  P  V  F  I  I  T  A  C  Y  L  T     240
676 GGC TTC CTG ATC CCC GTC TTC ATC ATC ACG GCC TGT TAC CTC ACC   720

241 I  V  F  K  L  Q  R  N  R  Q  A  K  T  K  K     255
721 ATC GTC TTC AAG TTG CAG CGC AAC CGC CAG GCC AAG ACC AAG AAG   765

256 P  F  K  I  I  I  T  I  I  I  T  F  F  L  C     270
766 CCC TTC AAG ATC ATC ATC ACC ATC ATC ATC ACC TTC TTC CTC TGC   810

271 W  C  P  Y  H  T  L  Y  L  L  E  L  H  H  T     285
811 TGG TGC CCC TAC CAC ACA CTC TAC CTG CTG GAG CTC CAC CAC ACG   855

286 A  V  P  A  S  V  F  S  L  G  L  P  L  A  T     300
```

Figure 4 Continued

```
 856 GCT GTG CCA GCC TCT GTC TTC AGC CTG GGA CTG CCC CTG GCC ACA  900

 301  A   V   A   I   A   N   S   C   M   N   P   I   L   Y   V  315
 901 GCC GTC GCC ATC GCC AAC AGC TGT ATG AAC CCC ATC CTG TAC GTC  945

 316  F   M   G   H   D   F   K   K   F   K   V   A   L   F   S  330
 946 TTC ATG GGC CAC GAC TTC AAA AAA TTC AAG GTG GCC CTT TTC TCC  990

 331  R   L   V   N   A   L   S   E   D   T   G   P   S   S   Y  345
 991 CGC CTG GTG AAT GCC CTG AGC GAG GAC ACA GGA CCC TCC TCC TAC 1035

 346  P   S   H   R   S   F   T   K   M   S   S   L   I   E   K  360
1036 CCC AGT CAC AGG AGC TTC ACC AAG ATG TCC TCA TTG ATT GAG AAG 1080

 361  A   S   V   N   E   K   E   T   S   T   L   *              372
1081 GCT TCA GTG AAT GAG AAA GAG ACC AGC ACC CTC TGA             1116
```

Figure 5: Alignment of ChemerinR

Alignment of the amino acid sequence of ChemerinR (ChemeR23) with AT2 receptors, C3a, C5a and fMLP receptor and other chemoattractants related sequences were performed using ClustalX algorithm. Then, the dendrogram was constucted using TreeView algorithm.

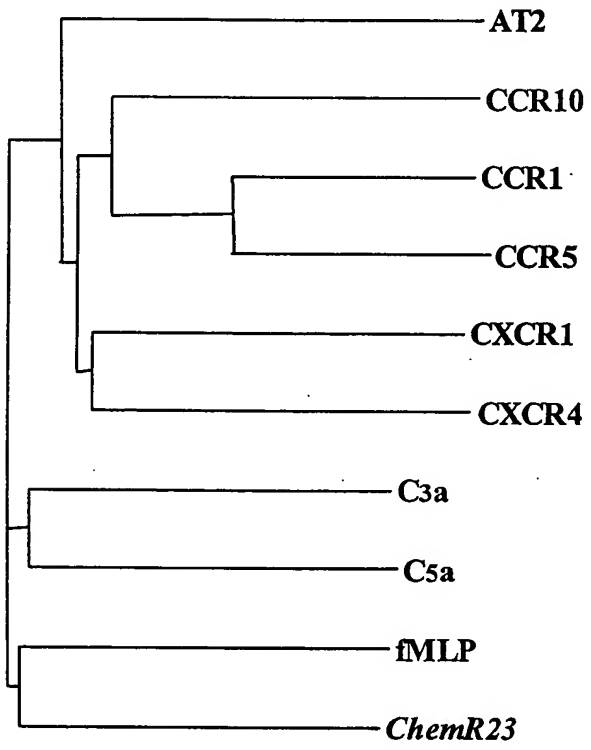


Figure 6: Nucleotide and deduced amino acid sequence of human Preprochemerin (AC Q99969 - SEQ ID Nos: 7 and 8, respectively)

```
   1  M   R   R   L   L   I   P   L   A   L   W   L   G   A   V    15
  97 ATG CGA CGG CTG CTG ATC CCT CTG GCC CTG TGG CTG GGT GCG GTG   141

  16  G   V   G   V   A   E   L   T   E   A   Q   R   R   G   L    30
 142 GGC GTG GGC GTC GCC GAG CTC ACG GAA GCC CAG CGC CGG GGC CTG   186

  31  Q   V   A   L   E   E   F   H   K   H   P   P   V   Q   W    45
 187 CAG GTG GCC CTG GAG GAA TTT CAC AAG CAC CCG CCC GTG CAG TGG   231

  46  A   F   Q   E   T   S   V   E   S   A   V   D   T   P   F    60
 232 GCC TTC CAG GAG ACC AGT GTG GAG AGC GCC GTG GAC ACG CCC TTC   276

  61  P   A   G   I   F   V   R   L   E   F   K   L   Q   Q   T    75
 277 CCA GCT GGA ATA TTT GTG AGG CTG GAA TTT AAG CTG CAG CAG ACA   321

  76  S   C   R   K   R   D   W   K   K   P   E   C   K   V   R    90
 322 AGC TGC CGG AAG AGG GAC TGG AAG AAA CCC GAG TGC AAA GTC AGG   366

  91  P   N   G   R   K   R   K   C   L   A   C   I   K   L   G   105
 367 CCC AAT GGG AGG AAA CGG AAA TGC CTG GCC TGC ATC AAA CTG GGC   411

 106  S   E   D   K   V   L   G   R   L   V   H   C   P   I   E   120
 412 TCT GAG GAC AAA GTT CTG GGC CGG TTG GTC CAC TGC CCC ATA GAG   456

 121  T   Q   V   L   R   E   A   E   E   H   Q   E   T   Q   C   135
 457 ACC CAA GTT CTG CGG GAG GCT GAG GAG CAC CAG GAG ACC CAG TGC   501

 136  L   R   V   Q   R   A   G   E   D   P   H   S   F   Y   F   150
 502 CTC AGG GTG CAG CGG GCT GGT GAG GAC CCC CAC AGC TTC TAC TTC   546

 151  P   G   Q   F   A   F   S   K   A   L   P   R   S   *       164
 547 CCT GGA CAG TTC GCC TTC TCC AAG GCC CTG CCC CGC AGC TAA       588
```

Figure 7: Nucleotide and deduced amino acid sequence of mouse Preprochemerin (SEQ ID Nos: 9 and 10, respectively)

```
   1  M   K   C   L   L   I   S   L   A   L   W   L   G   T   V     15
 102 ATG AAG TGC TTG CTG ATC TCC CTA GCC CTA TGG CTG GGC ACA GTG    146

  16  G   T   R   G   T   E   P   E   L   S   E   T   Q   R   R     30
 147 GGC ACA CGT GGG ACA GAG CCC GAA CTC AGC GAG ACC CAG CGC AGG    191

  31  S   L   Q   V   A   L   E   E   F   H   K   H   P   P   V     45
 192 AGC CTA CAG GTG GCT CTG GAG GAG TTC CAC AAA CAC CCA CCT GTG    236

  46  Q   L   A   F   Q   E   I   G   V   D   R   A   E   E   V     60
 237 CAG TTG GCC TTC CAA GAG ATC GGT GTG GAC AGA GCT GAA GAA GTG    281

  61  L   F   S   A   G   T   F   V   R   L   E   F   K   L   Q     75
 282 CTC TTC TCA GCT GGC ACC TTT GTG AGG TTG GAA TTT AAG CTC CAG    326

  76  Q   T   N   C   P   K   K   D   W   K   K   P   E   C   T     90
 327 CAG ACC AAC TGC CCC AAG AAG GAC TGG AAA AAG CCG GAG TGC ACA    371

  91  I   K   P   N   G   R   R   R   K   C   L   A   C   I   K    105
 372 ATC AAA CCA AAC GGG AGA AGG CGG AAA TGC CTG GCC TGC ATT AAA    416

 106  M   D   P   K   G   K   I   L   G   R   I   V   H   C   P    120
 417 ATG GAC CCC AAG GGT AAA ATT CTA GGC CGG ATA GTC CAC TGC CCA    461

 121  I   L   K   Q   G   P   Q   D   P   Q   E   L   Q   C   I    135
 462 ATT CTG AAG CAA GGG CCT CAG GAT CCT CAG GAG TTG CAA TGC ATT    506

 136  K   I   A   Q   A   G   E   D   P   H   G   Y   F   L   P    150
 507 AAG ATA GCA CAG GCT GGC GAA GAC CCC CAC GGC TAC TTC CTA CCT    551

 151  G   Q   F   A   F   S   R   A   L   R   T   K   *            163
 552 GGA CAG TTT GCC TTC TCC AGG GCC CTG AGA ACC AAA TAA            590
```

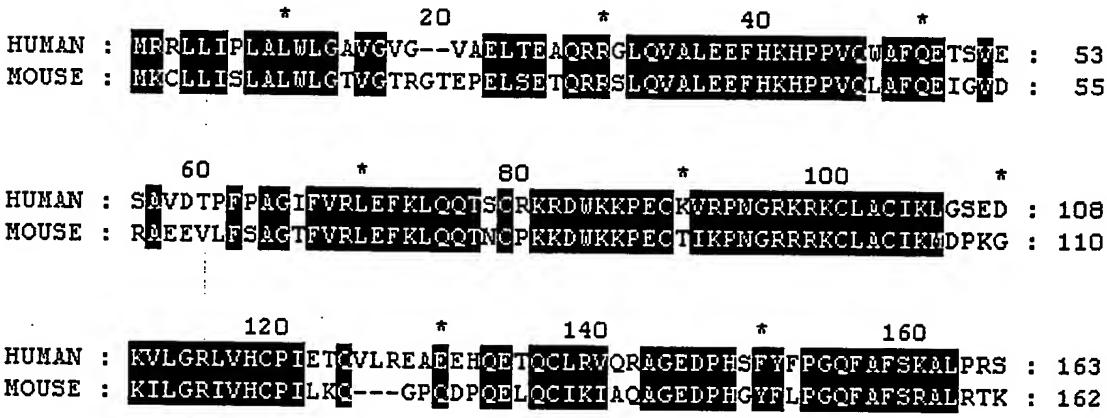
Figure 8: Nucleotide and deduced amino acid sequence of human Prochemerin (SEQ ID Nos: 11 and 12 respectively)

```
   1  E   L   T   E   A   Q   R   R   G   L   Q   V   A   L   E  15
   1 GAG CTC ACG GAA GCC CAG CGC CGG GGC CTG CAG GTG GCC CTG GAG  45

  16  E   F   H   K   H   P   P   V   Q   W   A   F   Q   E   T  30
  46 GAA TTT CAC AAG CAC CCG CCC GTG CAG TGG GCC TTC CAG GAG ACC  90

  31  S   V   E   S   A   V   D   T   P   F   P   A   G   I   F  45
  91 AGT GTG GAG AGC GCC GTG GAC ACG CCC TTC CCA GCT GGA ATA TTT 135

  46  V   R   L   E   F   K   L   Q   Q   T   S   C   R   K   R  60
 136 GTG AGG CTG GAA TTT AAG CTG CAG CAG ACA AGC TGC CGG AAG AGG 180

  61  D   W   K   K   P   E   C   K   V   R   P   N   G   R   K  75
 181 GAC TGG AAG AAA CCC GAG TGC AAA GTC AGG CCC AAT GGG AGG AAA 225

  76  R   K   C   L   A   C   I   K   L   G   S   E   D   K   V  90
 226 CGG AAA TGC CTG GCC TGC ATC AAA CTG GGC TCT GAG GAC AAA GTT 270

  91  L   G   R   L   V   H   C   P   I   E   T   Q   V   L   R 105
 271 CTG GGC CGG TTG GTC CAC TGC CCC ATA GAG ACC CAA GTT CTG CGG 315

 106  E   A   E   E   H   Q   E   T   Q   C   L   R   V   Q   R 120
 316 GAG GCT GAG GAG CAC CAG GAG ACC CAG TGC CTC AGG GTG CAG CGG 360

 121  A   G   E   D   P   H   S   F   Y   F   P   G   Q   F   A 135
 361 GCT GGT GAG GAC CCC CAC AGC TTC TAC TTC CCT GGA CAG TTC GCC 405

 136  F   S   K   A   L   P   R   S                             143
 406 TTC TCC AAG GCC CTG CCC CGC AGC                             429
```

Figure 9: Nucleotide and deduced amino acid sequence of human Chemerin (SEQ ID Nos 13 and 14 respectively)

```
   1  E   L   T   E   A   Q   R   R   G   L   Q   V   A   L   E  15
   1 GAG CTC ACG GAA GCC CAG CGC CGG GGC CTG CAG GTG GCC CTG GAG  45

  16  E   F   H   K   H   P   P   V   Q   W   A   F   Q   E   T  30
  46 GAA TTT CAC AAG CAC CCG CCC GTG CAG TGG GCC TTC CAG GAG ACC  90

  31  S   V   E   S   A   V   D   T   P   F   P   A   G   I   F  45
  91 AGT GTG GAG AGC GCC GTG GAC ACG CCC TTC CCA GCT GGA ATA TTT 135

  46  V   R   L   E   F   K   L   Q   Q   T   S   C   R   K   R  60
 136 GTG AGG CTG GAA TTT AAG CTG CAG CAG ACA AGC TGC CGG AAG AGG 180

  61  D   W   K   K   P   E   C   K   V   R   P   N   G   R   K  75
 181 GAC TGG AAG AAA CCC GAG TGC AAA GTC AGG CCC AAT GGG AGG AAA 225

  76  R   K   C   L   A   C   I   K   L   G   S   E   D   K   V  90
 226 CGG AAA TGC CTG GCC TGC ATC AAA CTG GGC TCT GAG GAC AAA GTT 270

  91  L   G   R   L   V   H   C   P   I   E   T   Q   V   L   R 105
 271 CTG GGC CGG TTG GTC CAC TGC CCC ATA GAG ACC CAA GTT CTG CGG 315

 106  E   A   E   E   H   Q   E   T   Q   C   L   R   V   Q   R 120
 316 GAG GCT GAG GAG CAC CAG GAG ACC CAG TGC CTC AGG GTG CAG CGG 360

 121  A   G   E   D   P   H   S   F   Y   F   P   G   Q   F   A 135
 361 GCT GGT GAG GAC CCC CAC AGC TTC TAC TTC CCT GGA CAG TTC GCC 405

 136  F   S                                                     137
 406 TTC TCC                                                     411
```

Figure 10: Amino acid sequence alignment of human (SEQ ID NO: 8) and mouse Preprochemerin (SEQ ID NO: 10). Identical and similar residues



are shaded.

Figure 11. Sequence Alignment of Chemerin Polypeptide Sequences

```
1                                                    50

   mus  MKCLLISLAL WLGTVGTRGT EPELSETQRR SLQVALEEFH KHPPVQLAFQ
   rat  MKCLLISLAL WLGTADIHGT ELELSETQRR GLQVALEEFH RHPPVQWAFQ
 human  MRRLLIPLAL WLGAVGV..G VAELTEAQRR GLQVALEEFH KHPPVQWAFQ
   sus  MWQLLLPLAL WLGTMGL..G RAELTAAQLR GLQVALEEFH KHPPVQWAFR
   bos  MWQLLLPLAL GLGTMGL..G RAELTTAQHR GLQVALEEFH KHPPVLWAFQ
gallus  ~RAVGMKLLL GIAVVVLALA DAGQSPLQRR VVKDVLDYFH SRSNVQFLFR

         51                                              100

   mus  EIGVDRAEEV LFSAGTFVRL EFKLQQTNCP KKDWKKPECT IKPNGRRRKC
   rat  EIGVDSADDL FFSAGTFVRL EFKLQQTSCL KKDWKKPECT IKPNGRKRKC
 human  ETSVESAVDT PFPAGIFVRL EFKLQQTSCR KRDWKKPECK VRPNGRKRKC
   sus  ETGVNSAMDT PFPAGTFVRL EFKLQQTSCR KRDWKKAECK VKPNGRKRKC
   bos  VTSVDNAADT LFPAGQFVRL EFKLQQTSCR KKDWRKEDCK VKPNGRKRKC
gallus  EQSVEGAVER VDSSGTFVQL HLNLAQTACR KQAQRKQNCR IMENRRKPVC

         101                                             150

   mus  LACIKMDPKG ..KILGRIVH C.PILKQGP. Q..DPQELQC IKIAQAGEDP
   rat  LACIKLDPKG ..KVLGRMVH C.PILKQGPQ Q..EPQESQC SKIAQAGEDS
 human  LACIKLGSED ..KVLGRLVH C.PIETQVLR EAEEHQETQC LRVQRAGEDP
   sus  LACIKLNSED ..KVLGRMVH C.PIETQVQR EPEERQEAQC SRVERAGEDP
   bos  LACIKLDSKD ..QVLGRMVH C.PIQTQVQR ELDDAQDAQC SRVERAGEDP
gallus  LACYKFDSSD VPKVLDKYYN CGPSHHLAMK DIKHRDEAEC RAVEEAGKTS
```

Figure 11 Continued

```
        151             168
   mus  HGYFLPGQFA FSRALRTK (SEQ ID NO: 10)
   rat  RIYFFPGQFA FSRAL (SEQ ID NO: 76)
 human  HSFYFPGQFA FSKALPRS (SEQ ID NO: 8)
   sus  HSYYFPGQFA FFKALPPS (SEQ ID NO: 77)
   bos  HSYYLPGQFA FIKAL (SEQ ID NO: 78)
gallus  DVLYLPGMFA FSKGLP (SEQ ID NO: 79)
```

Identities :

	bos.pep	mus.pep	sus.pep	gallus	rat.pep
human.pep	83.750	56.250	86.503	30.675	61.392
bos.pep		54.375	87.500	31.875	56.329
mus.pep			54.375	31.677	73.125
sus.pep				31.288	58.228
gallus.pep					30.818

Figure 12: Partial chromatogram of the fifth step of purification
The active fractions (approximately 28% CH3CN) of the previous step were diluted 6 fold with H_2O/0.1% TFA and directly loaded onto a C18 reverse phase column (1mm x 50 mm, Vydac) pre-equilabreted with 5% CH3CN/0.1% TFA at a flow-rate of 0.1 ml/min. at room temperature. A 5-95% gradient of CH3CN in 0.1%TFA was applied with a 0.3%/min slope between 25 and 45%. The activity was eluted at 40% CH3CN (indicated by the black horizontal line).

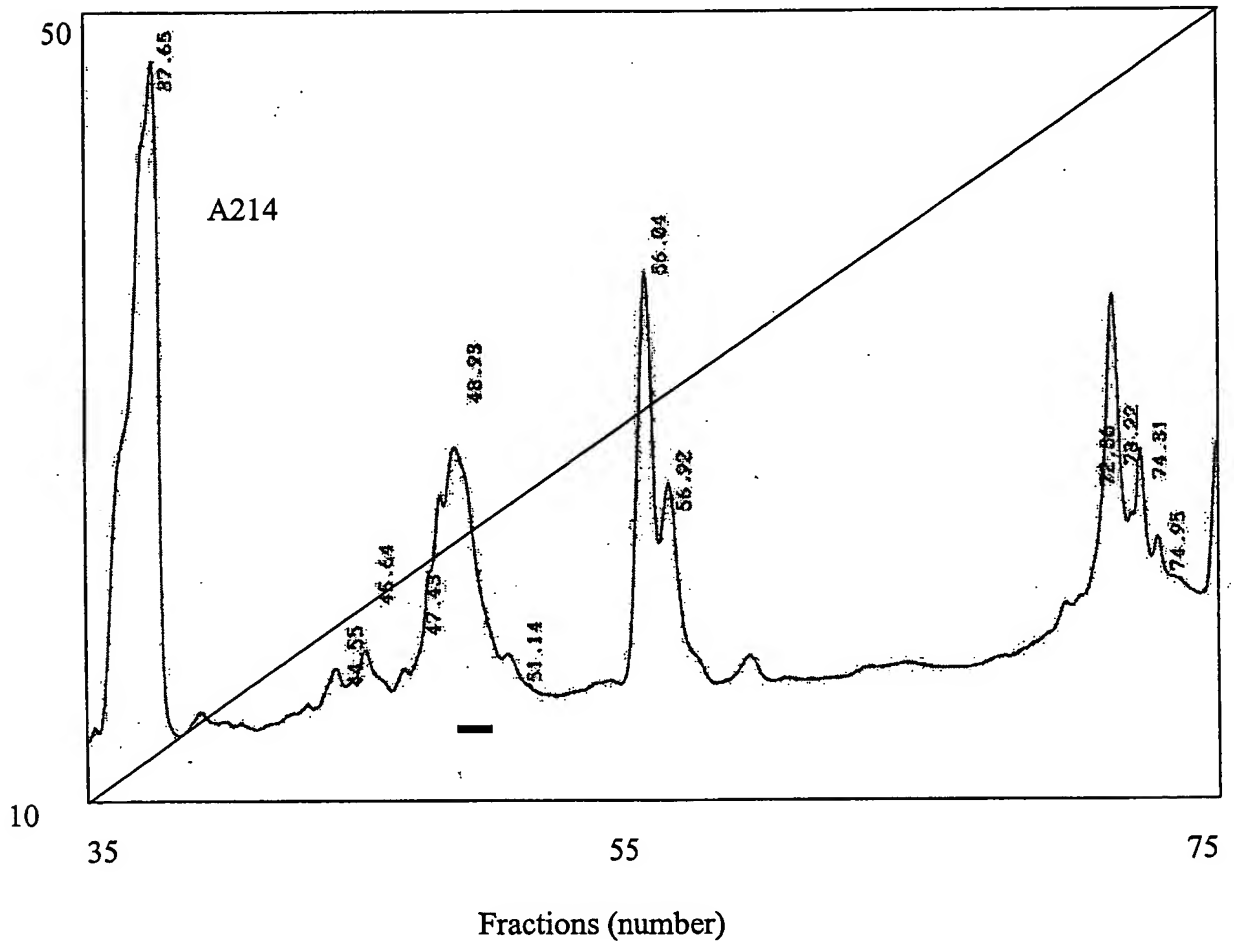


Figure 13: Primary screening of HPLC fractions obtained from the fractionation of human ovary ascites.

The different fractions obtained following fractionation of human ovary ascites were diluted fivefold in the buffer assay and tested in aequorin assay using a cell line expressing ChemerinR (open circles) or cell lines expressing not related receptors (closed triangles and squares). The response obtained for each fraction was normalized using the ATP response of each cell line.

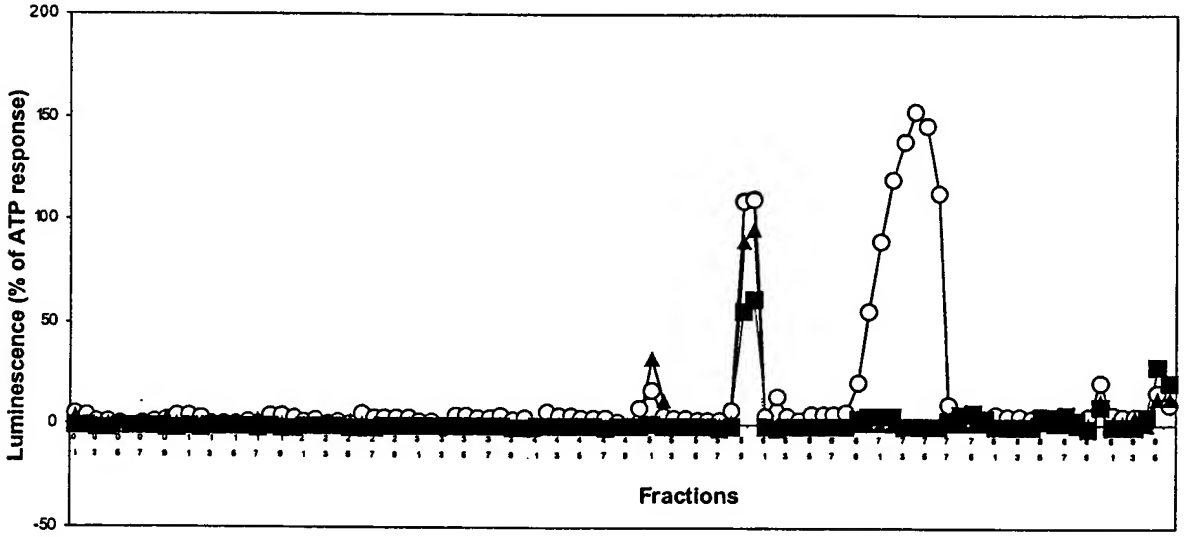


Figure 14: Activation of ChemerinR by cells transfected with Preprochemerin (TIG 2)

293 T cells were transiently transfected with pCDNA3- Preprochemerin (TIG2) or with pCDNA3 alone (mock transfected). Increasing volumes of the supernatant collected 4 days following transfection were analysed in a aequorin-based assay with CHO cells expressing ChemerinR. A representative experiment is shown. Assay was performed in triplicate and SD are indicated.

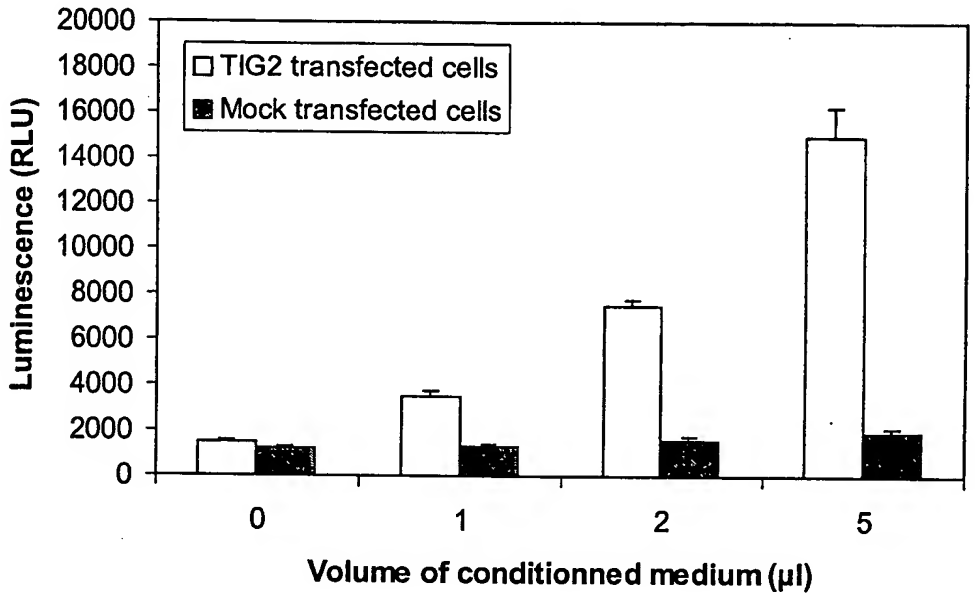


Figure 15: Characterization of antibodies directed against ChemerinR

A mixture of recombinant cells made up of 2/3 recombinant ChemerinR CHO cells and 1/3 recombinant HCR CHO cells (negative control) was subject to react with either a supernatant of the anti ChemerinR 5C 1H2 monoclonal antibody (thick line) or a supernatant with no known antibody activity (thin line, grey filling). After staining with FITC labeled anti mouse Ig these preparations were analysed by flow cytofluorometry. Results are displayed as a histogram of the number of cells (Events axis) expressing a given fluorescence (FL1-H axis). Monoclonal 5C 1H2 allowed to discriminate the ChemerinR recombinant sub-population of cells from the negative control cells as evidenced by the relative proportions of both type of cells. The background fluorescence of the assay is given by the second staining (grey filling).

Figure 16.

Nucleotide (SEQ ID NO: 72) and deduced amino acid sequence (SEQ ID NO: 73) of a human truncated form of Proprechemerin

```
  1  M   R   R   L   L   I   P   L   A   L   W   L   G   A   V      15
  1 ATG CGA CGG CTG CTG ATC CCT CTG GCC CTG TGG CTG GGT GCG GTG      45

 16  G   V   G   V   A   E   L   T   E   A   Q   R   R   G   L      30
 46 GGC GTG GGC GTC GCC GAG CTC ACG GAA GCC CAG CGC CGG GGC CTG      90

 31  Q   V   A   L   E   E   F   H   K   H   P   P   V   Q   W      45
 91 CAG GTG GCC CTG GAG GAA TTT CAC AAG CAC CCG CCC GTG CAG TGG     135

 46  A   F   Q   E   T   S   V   E   S   A   V   D   T   P   F      60
136 GCC TTC CAG GAG ACC AGT GTG GAG AGC GCC GTG GAC ACG CCC TTC     180

 61  P   A   G   I   F   V   R   L   E   F   K   L   Q   Q   T      75
181 CCA GCT GGA ATA TTT GTG AGG CTG GAA TTT AAG CTG CAG CAG ACA     225

 76  S   C   R   K   R   D   W   K   K   P   E   C   K   V   R      90
226 AGC TGC CGG AAG AGG GAC TGG AAG AAA CCC GAG TGC AAA GTC AGG     270

 91  P   N   G   R   K   R   K   C   L   A   C   I   K   L   G     105
271 CCC AAT GGG AGG AAA CGG AAA TGC CTG GCC TGC ATC AAA CTG GGC     315

106  S   E   D   K   V   L   G   R   L   V   H   C   P   I   E     120
316 TCT GAG GAC AAA GTT CTG GGC CGG TTG GTC CAC TGC CCC ATA GAG     360

121  T   Q   V   L   R   E   A   E   E   H   Q   E   T   Q   C     135
361 ACC CAA GTT CTG CGG GAG GCT GAG GAG CAC CAG GAG ACC CAG TGC     405

136  L   R   V   Q   R   A   G   E   D   P   H   S   F   Y   F     150
406 CTC AGG GTG CAG CGG GCT GGT GAG GAC CCC CAC AGC TTC TAC TTC     450

151  P   G   Q   F   A   F   S                                     157
451 CCT GGA CAG TTC GCC TTC TCC                                     471
```

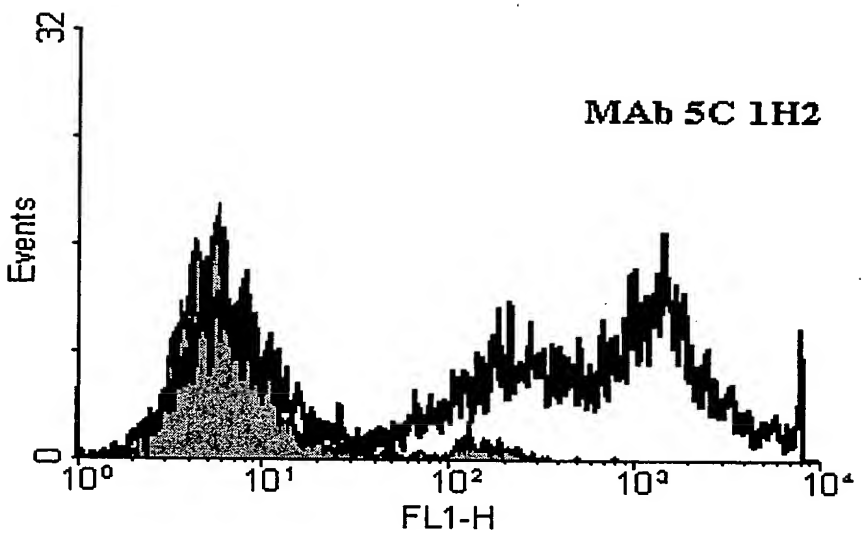


Figure 16 Continued

Figure 17

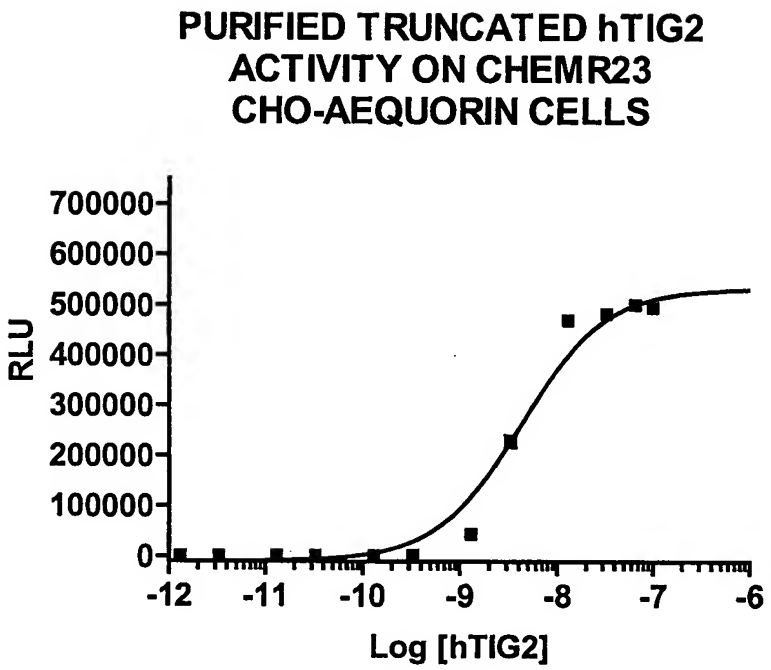

PURIFIED TRUNCATED hTIG2
ACTIVITY ON CHEMR23
CHO-AEQUORIN CELLS
RLU
700000
600000
500000
400000
300000
200000
100000
0
-12
-11
-10
-9
-8
-7
-6
Log [hTIG2]

Figure 18.

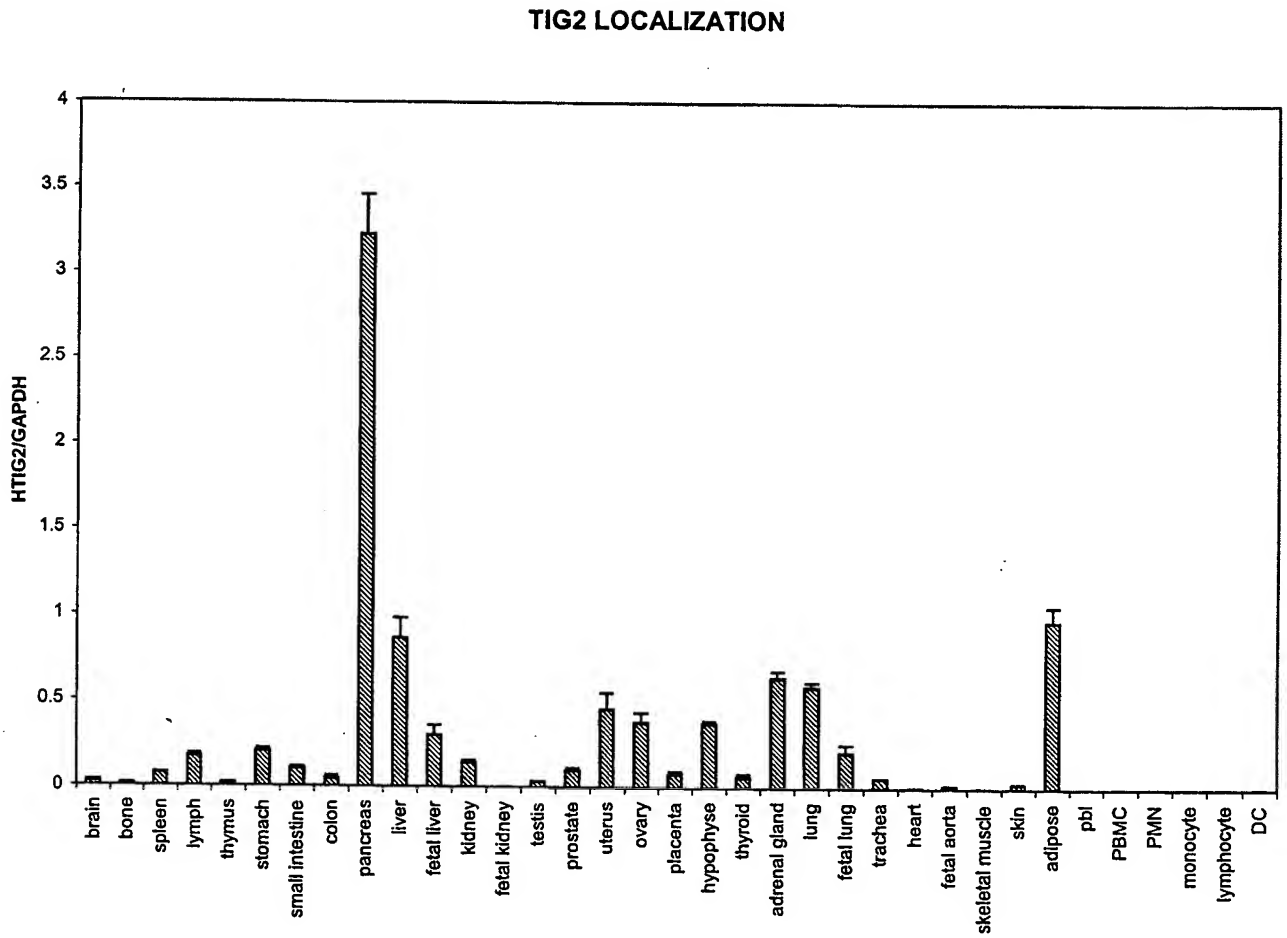

TIG2 LOCALIZATION
HTIG2/GAPDH
4
3.5
3
2.5
2
1.5
1
0.5
0
brain
bone
spleen
lymph
thymus
stomach
small intestine
colon
pancreas
liver
fetal liver
kidney
fetal kidney
testis
prostate
uterus
ovary
placenta
hypophyse
thyroid
adrenal gland
lung
fetal lung
trachea
heart
fetal aorta
skeletal muscle
skin
adipose
pbl
PBMC
PMN
monocyte
lymphocyte
DC

Figure 19

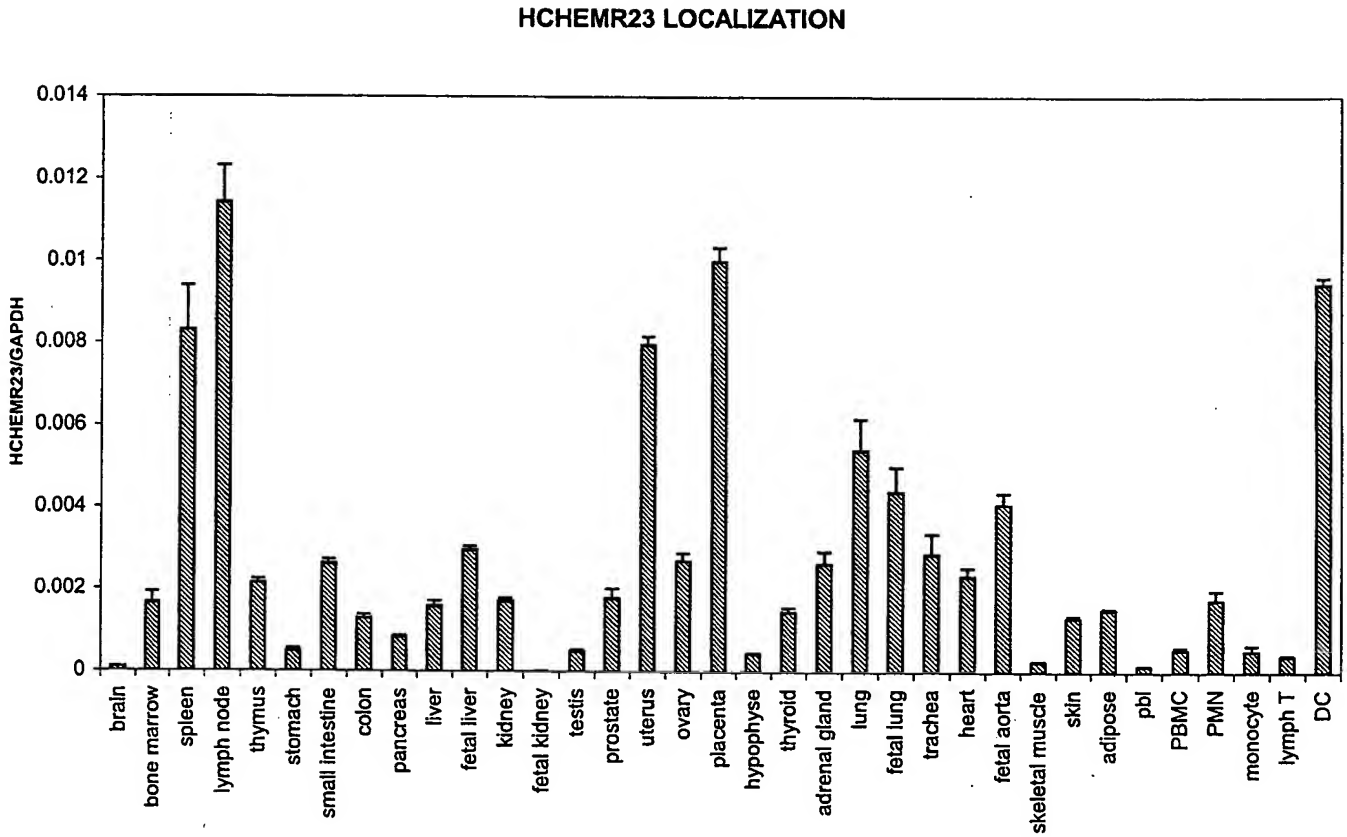

HCHEMR23 LOCALIZATION
HCHEMR23/GAPDH
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
brain
bone marrow
spleen
lymph node
thymus
stomach
small intestine
colon
pancreas
liver
fetal liver
kidney
fetal kidney
testis
prostate
uterus
ovary
placenta
hypophyse
thyroid
adrenal gland
lung
fetal lung
trachea
heart
fetal aorta
skeletal muscle
skin
adipose
pbl
PBMC
PMN
monocyte
lymph T
DC

Figure 20a Human Chemerin peptides

Human prochemerin-25	QRAGEDPHSFYFPGQFAFSKALPRS
Human prochemerin-6	KALPRS
Human Chemerin-19	QRAGEDPHSFYFPGQFAFS
Human [Lys-20]Chemerin-19	QRAGEDPHSFYFPGQFAFSK
Human [ΔSer19]Chemerin-19	QRAGEDPHSFYFPGQFAF
Human [ΔPhe18Ser19]Chemerin-19	QRAGEDPHSFYFPGQFA
Human Chemerin-17	AGEDPHSFYFPGQFAFS
Human Chemerin-15	EDPHSFYFPGQFAFS
Human Chemerin-13	PHSFYFPGQFAFS
Human Chemerin-12	HSFYFPGQFAFS
Human Chemerin-11	SFYFPGQFAFS
Human Chemerin-10	FYFPGQFAFS
Human Chemerin-9	YFPGQFAFS
Human Chemerin-8	FPGQFAFS
Human Chemerin-7	PGQFAFS
Human Chemerin-6	GQFAFS
Human Chemerin-5	QFAFS
Human [Ala-1]Chemerin-9	AFPGQFAFS
Human [Ala-2]Chemerin-9	YAPGQFAFS
Human [Ala-3]Chemerin-9	YFAGQFAFS
Human [Ala-4]Chemerin-9	YFPAQFAFS
Human [Ala-5]Chemerin-9	YFPGAFAFS
Human [Ala-6]Chemerin-9	YFPGQAAFS
Human [Ala-8]Chemerin-9	YFPGQFAAS
Human [Ala-9]Chemerin-9	YFPGQFAFA

Figure 20b Mouse Chemerin polypeptides

Mouse Chemerin-19	AQAGEDPHGYFLPGQFAFS (SEQ ID NO: 43)
Mouse Chemerin-12	HGYFLPGQFAFS (SEQ ID NO: 44)
Mouse Chemerin-11	GYFLPGQFAFS (SEQ ID NO: 45)
Mouse Chemerin-10	YFLPGQFAFS (SEQ ID NO: 46)
Mouse Chemerin-9	FLPGQFAFS (SEQ ID NO: 47)
Mouse Chemerin-8	LPGQFAFS (SEQ ID NO: 48)
Mouse prochemerin-26	IAQAGEDPHGYFLPGQFAFSRALRTK (SEQ ID NO: 49)
Mouse [Arg-21]Chemerin-20	IAQAGEDPHGYFLPGQFAFSR (SEQ ID NO: 50)

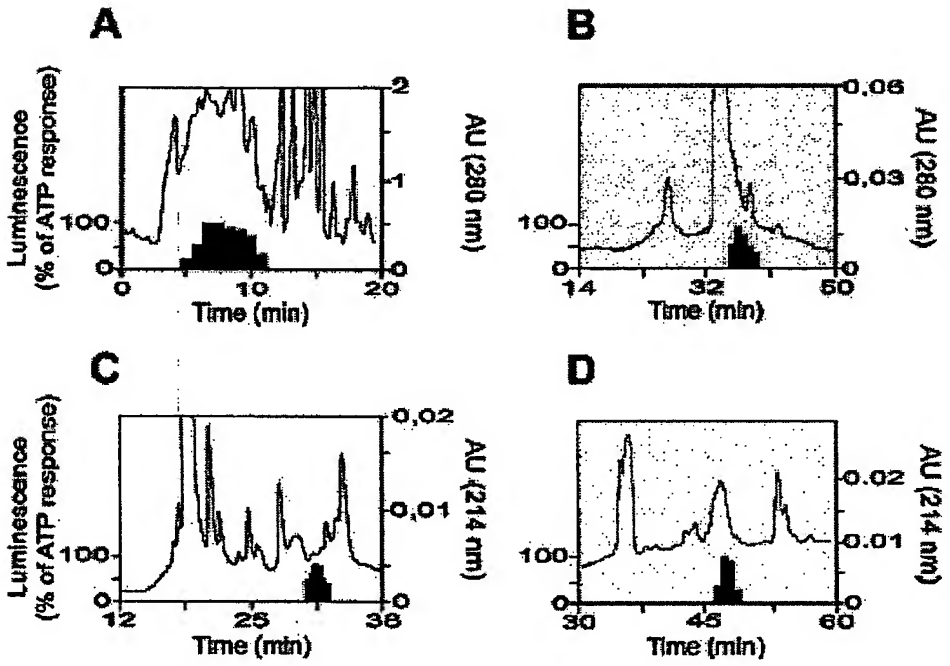
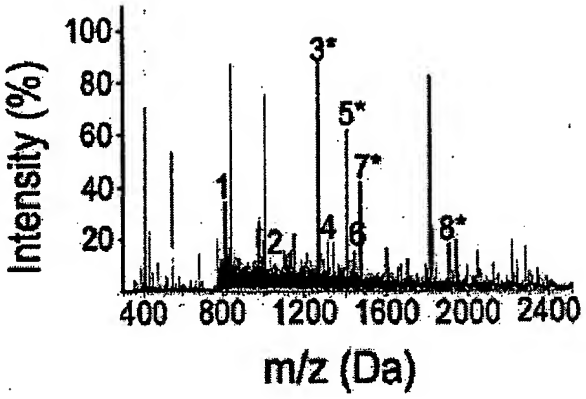


Figure 21. Purification of the natural ligand of the ChemR23 receptor from human inflammatory fluid. A, First step HPLC fractionation (Poros column) of human ascitic fluid. The absorbance (AU) and biological activity on ChemR23 (luminescence in an aequorin-based assay, normalized to the ATP response, black bars) are shown. **B**, Third step (cation-exchange column). **C**, Fourth step (C18 column). **D**, Last step purification of the active compound (C18 column). The X axis is zoomed to focus on the region of interest.

Figure 22

A



B

#	a.a.	Sequence	M+H
1	72-78	(K) LQQTSCR (K)	835.41
2	81-88	(R) DWKKPECK (V)	1033.51
3*	29-39	(R) GLQVALEEFHK (H)	1270.68
4	98-109	(K) CLACIKLGSEDK (V)	1279.64
5*	114-125	(R) LVHCPIETQVLR (E)	1407.78
6	28-39	(R) RGLQVALEEFHK (H)	1426.78
7*	126-137	(R) EAEEHQETQCLR (V)	1472.64
8*	141-157	(R) AGEDPHSFYFPGQFAFS (K)	1904.02

C

Figure 22. Identification of Chemerin as the natural ligand of ChemR23, the Chemerin receptor. A, Monoisotopic peptide mass fingerprinting of the active fraction on a Maldi Q-TOF mass spectrometer following trypsin digestion. **B**, Sequences corresponding to selected major peaks of the Maldi Q-TOF mass spectrometer spectrum following trypsin digestion. Peptides 1-7 correspond to tryptic peptides derived from the TIG-2 gene product (prochemerin), while peptide 8 is not tryptic and corresponds to the C-terminal end of the purified protein. The position of the peptides within this sequence is given. The sequence of peptides in peaks 3, 5, 7 and 8 was confirmed by microsequencing. **C**, Amino acid sequence alignment of human (SEQ ID NO: 8) and mouse (accession number: AK002298, SEQ ID NO: 10) preprochemerin, and human cathelicidin FALL39 (SEQ ID NO: 51) precursor. Aminoacid identities as compared to human preprochemerin are boxed. The signal peptides (predicted for mouse preprochemerin) are in bold lowercase characters, cysteines are in bold. Cleaved C-terminal peptides are in bold italics and underlined (predicted by analogy for mouse prochemerin). The location of introns (that interrupt the gene coding sequences between codons) are indicated by arrowheads.

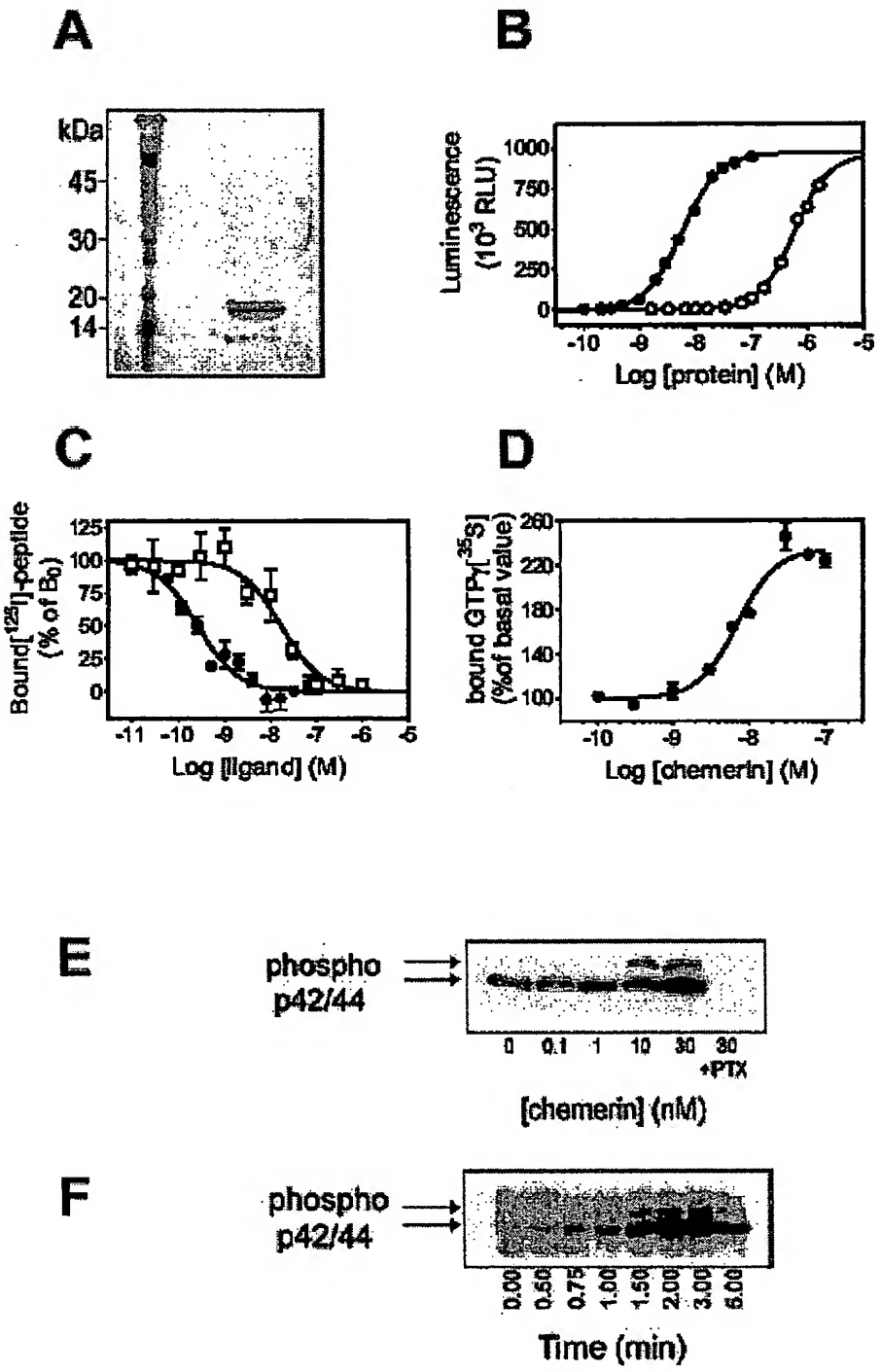


Figure 23. Pharmacology of the Chemerin receptor. **A**, SDS/PAGE of humanrecombinant Chemerin, expressed in CHO-K1 cells and purified by HPLC. The gel was silver stained and the major band corresponds to a protein of 18 kDa.Mass spectrometry analysis demonstrated the cleavage of the six C-terminal amino acids in this biologically active protein. **B**, Biological

Figure 23 Continued

activity on ChemerinR of human recombinant Chemerin (filled circles) and prochemerin (open circles),using the aequorin assay. **C**, Competition binding assay using as tracer an iodinated peptide derived from the Chemerin C-terminus. Competition was performed with the unlabeled peptide (open squares) or human recombinant Chemerin (filled circles). **D**, Concentration-action curve of human Chemerin in a GTP [^{35}S]-binding assay, using membranes of CHO/ChemerinR cells. **E**, Immunodetection of phosphorylated ERK1/2 in CHO/ChemerinR cells, following stimulation by human recombinant Chemerin for 2 min. **F**, Kinetics of ERK1/ ERK2 activation following stimulation by 10 nM human Chemerin. Each experiment was repeated at least three times.

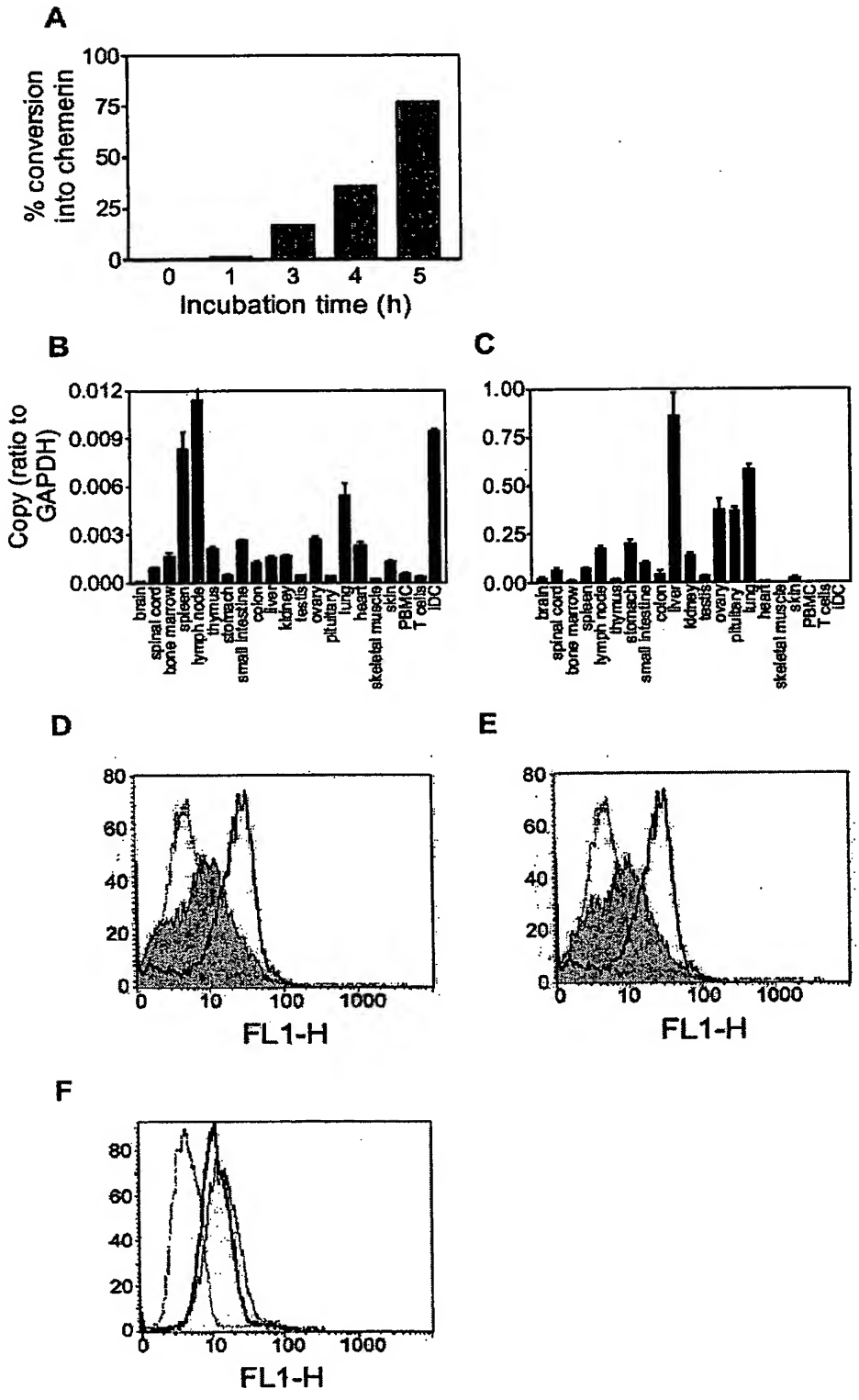


Figure 24. Expression of human Chemerin and its receptor. A, Conversion of human recombinant prochemerin (100 nM) in conditioned medium from hamster CHO-K1 cells. Conversion rate was estimated by comparing the biological activity with that of the same molar

Figure 24 Continued

amount of purified processed Chemerin. **B** and **C**, Transcripts encoding human ChemerinR (**B**) and prochemerin (**C**) were amplified by quantitative RT-PCR in a set of human tissues and cell populations. PBMC : peripheral blood mononuclear cells, iDC : immature dendritic cells. **D** and **E**, The expression of ChemerinR was analyzed by FACS in immature (solid line) and mature dendritic cells (gray area), following stimulation by LPS (**D**) or CD40L (**E**), using the 1H2 monoclonal antibody (IgG2A). Control labeling (dotted line) was made with an antibody of the same isotype. **F**, ChemerinR expression on macrophages was monitored using the 1H2 (thick solid line) and 4C7 (thin solid line) monoclonal antibodies. Control labeling (dotted line) was made with an antibody of the same isotype.

FIGURE1

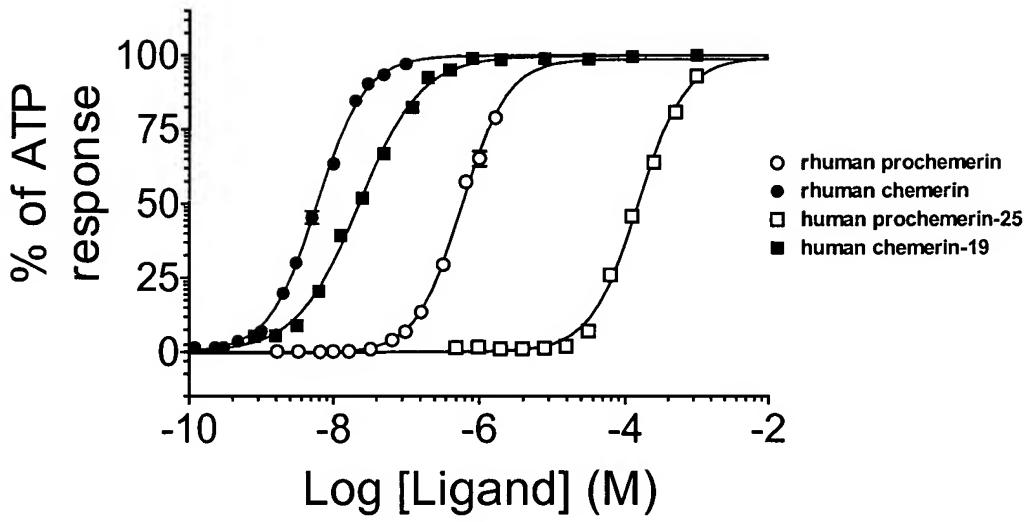


Figure 25A. Biological activity of Chemerin and C-terminal peptides on ChemR23. The biological activity of human recombinant prochemerin , human recombinant processed Chemerin, a 25 amino-acid C-terminal peptide of prochemerin, the corresponding 19 amino-acid C-terminal peptide of processed Chemerin, on human ChemR23 expressed in a CHO-K1 cell line, using the aequorin-based intracellular Ca^{2+} release assay (aequorin assay).

FIGURE2

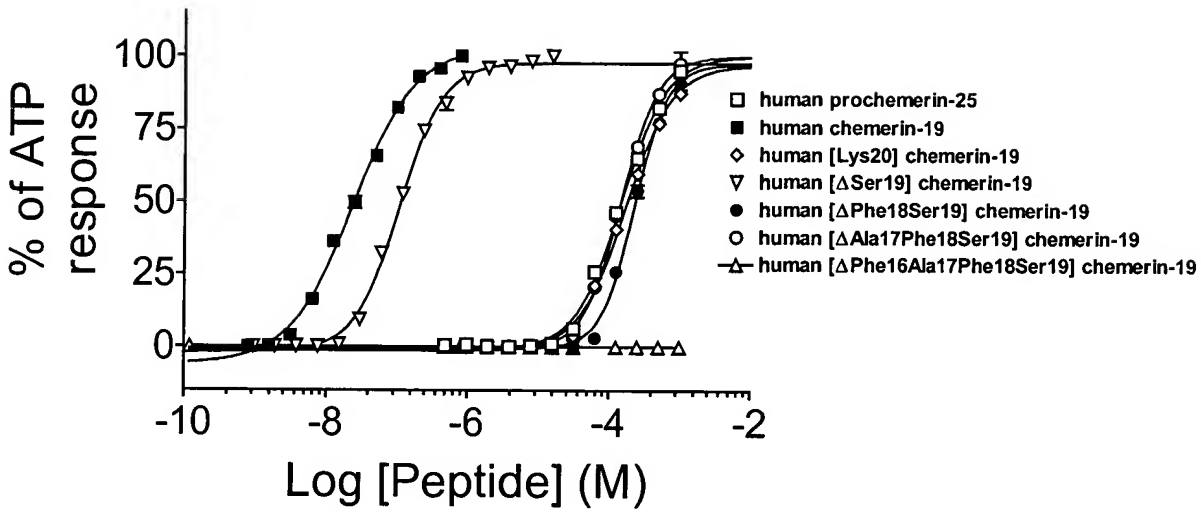


Figure 25B. Effect of C-terminal truncation on Chemerin biological activity. Biological activity of peptides C-terminally extended or truncated as compared to the C-terminus of processed Chemerin. (human Chemerin-19) on human ChemR23 expressed in a CHO-K1 cell line, using the aequorin-based intracellular Ca^{2+} release assay (aequorin assay)

FIGURE3

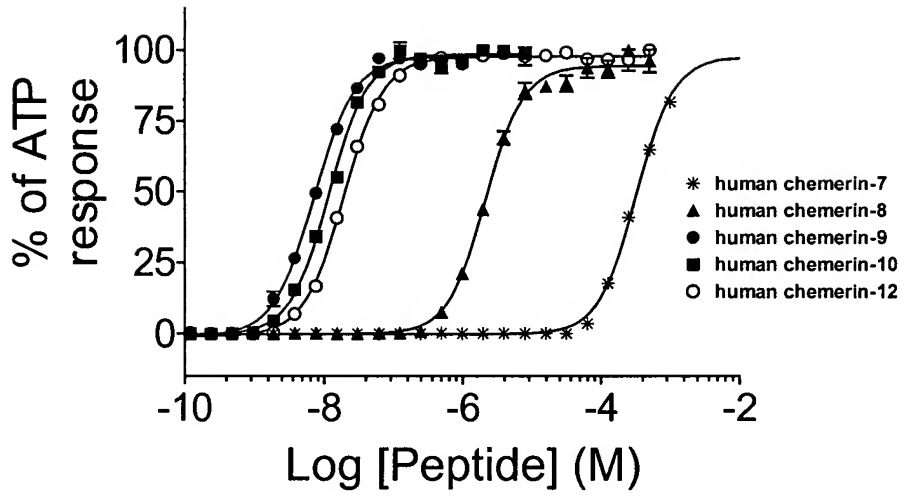


Figure 25C. Effect of N-terminal truncation on the biological activity of Chemerin-derived peptides. Biological activity of peptides N-terminally truncated as compared to human Chemerin-19 on human ChemR23 expressed in a CHO-K1 cell line, using the aequorin-based intracellular Ca^{2+} release assay (aequorin assay).

FIGURE 4

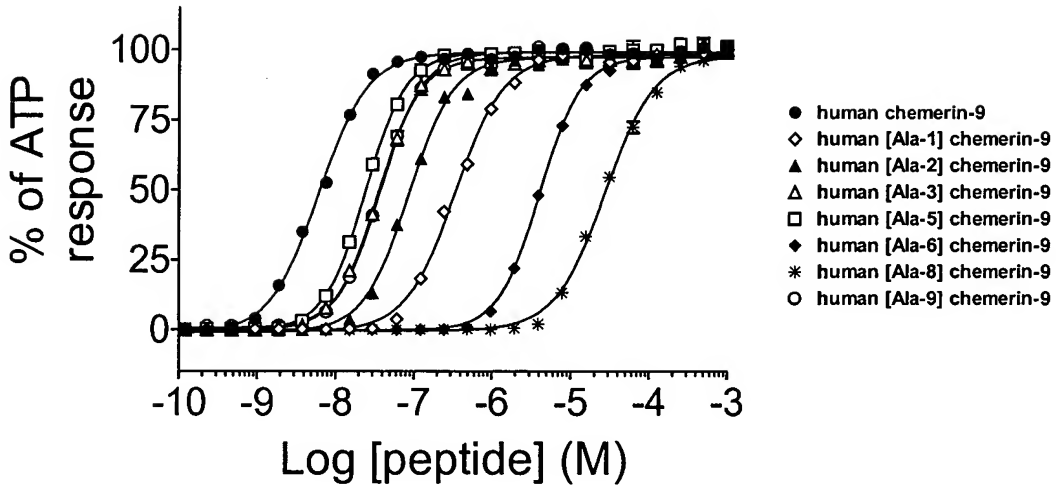
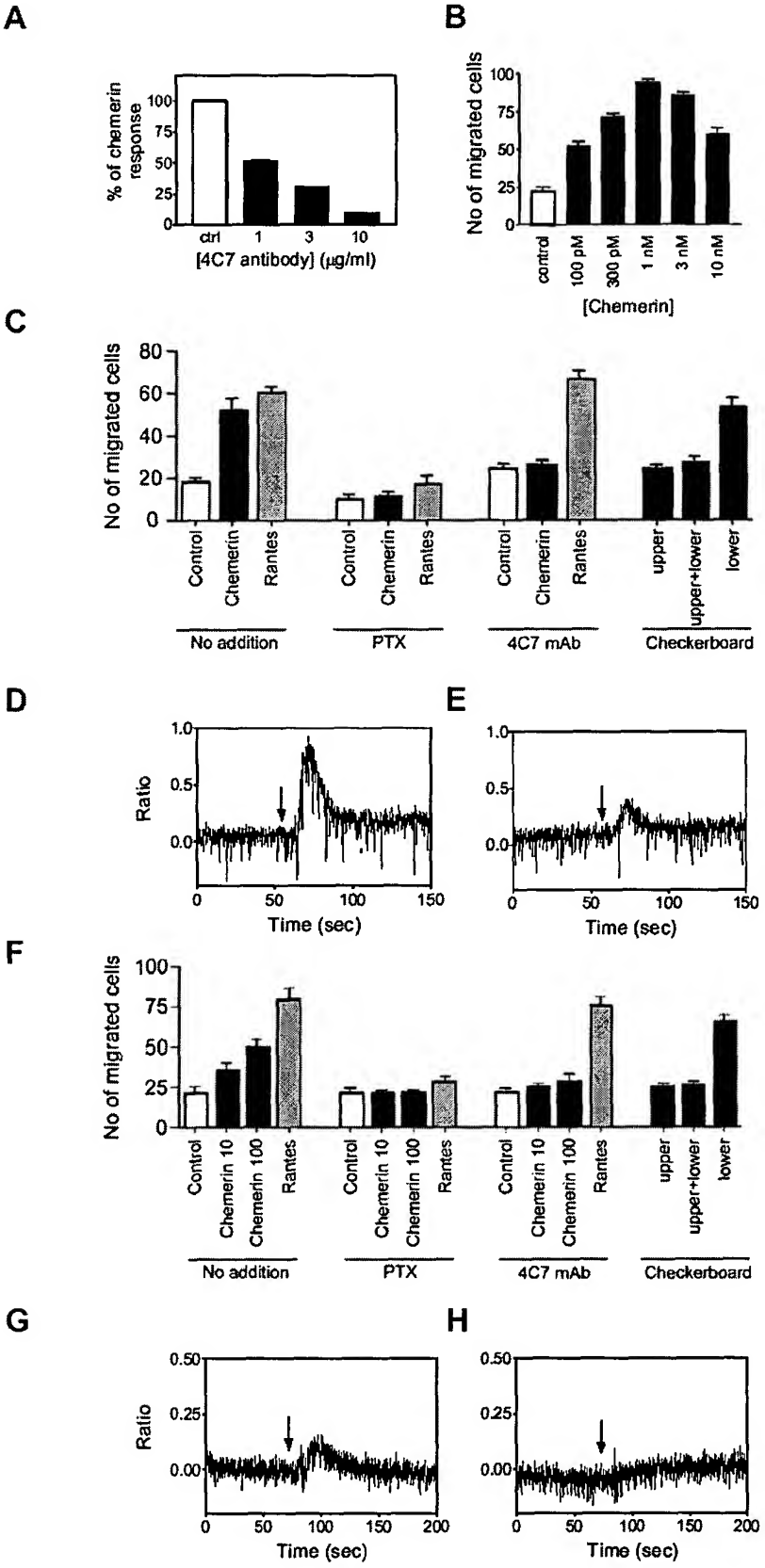


Figure 25D. Alanine scan of the Chemerin-9 peptide. Biological activity of peptides representing an ala-scan of the shorter C-terminal peptide (Chemerin-9) displaying an almost full activity on human ChemR23 expressed in a CHO-K1 cell line, using the aequorin-based intracellular Ca^{2+} release assay (aequorin assay).

Figure 26. Biological activity of Chemerin on primary cells. **A**, Inhibition of the functional response of CHO-K1 cells expressing the ChemerinR (aequorin assay) by the 4C7 anti-ChemerinR monoclonal antibody. The cells were preincubated for 30 min at room temperature with various amounts of the 4C7 antibody before stimulation by 10 nM recombinant Chemerin. The data were normalized according to the response in the absence of antibody (100%) and in the absence of agonist (0%). **B**, Chemotaxis of human immature dendritic cells by recombinant Chemerin. Results are expressed as the mean ± s.d. (n = 3), and are representative of three donors. **C**, Chemerin-induced (10 pM) dendritic cell migration was inhibited by pertussis toxin (3 μg/ml) pretreatment of the cells, as well as by preincubation of the cells with the 4C7 monoclonal antibody (10 μg/ml). Checkerboard analysis investigates chemotactic versus chemokinetic effects of Chemerin on dendritic cells. Human Chemerin (10 pM) was added to the lower and/or upper chamber of the chemotaxis device. The chemokine RANTES (10 nM) was used as a positive control in the experiments. **D**, Ca^{2+} flux in monocyte-derived dendritic cells in response to recombinant Chemerin (30 nM, arrow). **E**, The same experiment after 30 min preincubation of the cells with the 4C7 monoclonal antibody (10 μg/ml). **F**, Chemerin-induced macrophage migration (10 and 100 pM) and its inhibition by Pertussis toxin (3 μg/ml) pretreatment and 4C7 monoclonal antibody (10 μg/ml). Checkerboard analysis investigates chemotactic versus chemokinetic effects of Chemerin on macrophages. **G**, Ca^{2+} flux in macrophages in response to recombinant Chemerin (30 nM, arrow). **H**, The same experiment after 30 min preincubation of the cells with the 4C7 monoclonal antibody (10 μg/ml).

Figure 26


A
% of chemerin response
100
75
50
25
0
ctrl
1
3
10
[4C7 antibody] (μg/ml)
B
No of migrated cells
100
75
50
25
0
control
100 pM
300 pM
1 nM
3 nM
10 nM
[Chemerin]
C
No of migrated cells
80
60
40
20
0
Control
Chemerin
Rantes
Control
Chemerin
Rantes
Control
Chemerin
Rantes
upper
upper+lower
lower
No addition
PTX
4C7 mAb
Checkerboard
D
Ratio
1.0
0.5
0.0
0
50
100
150
Time (sec)
E
1.0
0.5
0.0
0
50
100
150
Time (sec)
F
No of migrated cells
100
75
50
25
0
Control
Chemerin 10
Chemerin 100
Rantes
Control
Chemerin 10
Chemerin 100
Rantes
Control
Chemerin 10
Chemerin 100
Rantes
upper
upper+lower
lower
No addition
PTX
4C7 mAb
Checkerboard
G
Ratio
0.50
0.25
0.00
0
50
100
150
200
Time (sec)
H
0.50
0.25
0.00
0
50
100
150
200
Time (sec)

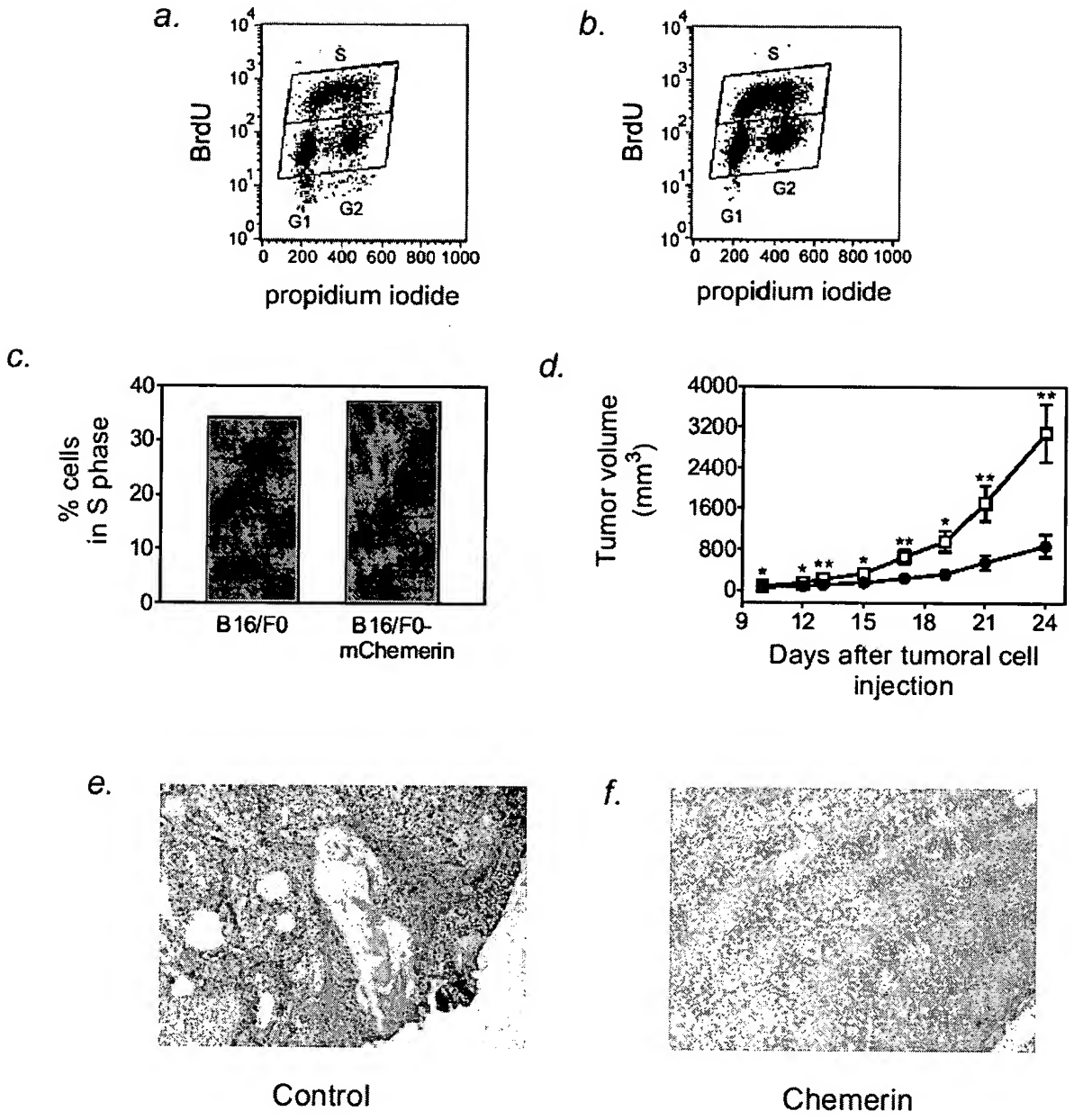


Figure 27. Anti-tumor activity of mouse Chemerin *in vivo*. A-C, Estimation of the proportion of cell population in G1, G2 and S phase following BrdU incorporation and propidium iodide staining. FACS analysis of control (A) and prochemerin-expressing B16/F0 (B) cells, and percentage of cells in S phase (C). D, Estimation size of tumors in mice, following the graft of B16/F0 cells expressing (filled circles) or not (open squares) mouse Chemerin. The data represent the mean ± s.e.m. for n=11 in each group, and are representative of three experiments performed independently with similar results. **:p<0.05,***: p<0.01, unpaired non parametric Mann-Whitney test. E and F, Hematoxylineosin staining of cryosections through control (E) and prochemerin-expressing (F) tumors, 18 days after the graft.